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MOLECULAR MAPPING OF THE FASCIATION MUTATION IN SOYBEAN, *GLYCINE MAX* (LEGUMINOSAE)[1]

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The spontaneous fasciation mutation generates novel developmental diversity in cultivated soybean, *Glycine max* (L.) Merrill. An increased apical dominance in the mutant inhibits axillary buds, causes a branchless phenotype, and restricts reproduction to shoot apices. The fasciation mutation is encoded by a recessive (*f*) allele at a single locus. The mutation, despite its importance in soybean development, has no locus assignment on previously reported molecular maps of soybean. A population of 70 F_2 progeny was derived from a cross between 'Clark 63' and the fasciation mutant. More than 700 molecular markers (amplified restriction fragment length polymorphisms [AFLPs], random amplified polymorphic DNAs [RAPDs], restriction fragment length polymorphisms [RFLPs], and simple sequence repeats [SSRs]) were used in mapping of the fasciation phenotype. Twenty linkage groups (LGs) corresponding to the public soybean molecular map are represented on the Clark 63 × fasciation mutant molecular map that spans 3050 centimorgans (cM). The *f* locus was mapped on LG D1b+W and linked with two AFLPs and four SSR markers (Satt005, Satt141, Satt600, and Satt703). No linkage was found between the *f* locus and several cDNA polymorphic loci between the wild type and the mutant. The known map position of the *f* locus and demonstration of the mutant phenotype from early postembryonic throughout reproductive stages provide an excellent resource for investigations of molecular mechanisms affecting soybean ontogeny.

Key words: development; fasciation; *Glycine max*; Leguminosae; map; molecular markers; mutant; polymorphism; soybean.

In soybean, *Glycine max* (L.) Merr., the fasciation mutation is a single gene trait encoded by a recessive allele (Nagai, 1926; Albertsen et al., 1983). The mutation has pleiotropic effects on plant development and pattern formation, and it changes phyllotaxy and plastochron of soybean plants (LaMotte et al., 1988; Tang and Knap, 1998). In early postembryonic development, the mutation causes enlargement of the shoot apex and its shape is altered from a dome to a ridge-like structure. Our previous studies suggest that the fasciation gene may be involved in balancing meristem maintenance and organ differentiation (Tang and Knap, 1998). The apical meristem of the mutant generates almost twice the number of leaves as the wild type plant, owing to meristem enlargement and rapid leaf primordia initiation. Strong apical dominance in the mutant suppresses development of axillary buds and causes a branchless phenotype. Floral production occurs at the shoot apices and results in a pod-set pattern resembling cauliflower. The drastic developmental changes caused by the fasciation allele do not result in a substantial reproductive penalty for the mutant plants (Albertsen et al., 1983; Wongyai, Tadahiko, and Matsumoto, 1984).

Domestication of plants seems to favor an increase in apical dominance during the evolution of cultivated species. In the domestication of maize, *Zea mays* subsp. *mays*, there is a profound increase in apical dominance relative to its wild ancestor teosinte, *Zea mays* subsp. *parviglumi* (Doebley, Stec, and Hubbard, 1997; Lukens and Doebley, 2001). The importance of branching may carry evolutionary implications. In this context, contribution of the apical meristem to plant architecture may prove to be informative about the evolution of morphological characters in plants. The strong dominance of the apical meristem is the most characteristic trait of the fasciation mutant in soybean and generates developmental diversity useful for investigation of genetic and hormonal factors important for plant architecture.

The frequent occurrence of the fasciation mutations in many different species (spontaneous fasciation mutations have been reported in more than 100 vascular plant families; Kiesselbach, 1926; White, 1945; Zielinski, 1945) suggests that the regulatory factors, which are fundamental to plant development, must be affected in individual mutants. Characterization of *Arabidopsis thaliana* fasciation mutants implicated several genes including *CLAVATA1* (Clark, Running, and Meyerowitz, 1993; Clark, Williams, and Meyerowitz, 1997); *CLAVATA2* (Kayes and Clark, 1998); *CLAVATA3* (Clark, Running, and Meyerowitz, 1995); *FASCIATA1* and *FASCIATA2* (Leyser and Furner, 1992; Kaya et al., 2001). Changes in the function of these genes lead to developmental alterations resulting in a similar fasciation phenotype (Wilkinson and Haughn, 1995; Meyerowitz, 1997; Laufs et al., 1998). In our previous studies, two *GmCLV1* genes were isolated from soybean (Yamamoto, Karakaya, and Knap, 2000). Expression and sequencing analysis showed that *GmCLV1A* and *GmCLV1B* were not responsible for the fasciation mutation. The *GmCLV1A* locus maps to linkage group (LG) H of the soybean molecular map (Yamamoto, Karakaya, and Knap, 2000). Also, sequencing and expression analysis of a soybean ortholog of the *Arabidopsis FASCIATA2* gene excluded that gene as a candidate for the fasciation mutation in soybean (Karakaya, 2001).

The genetic approach can provide a valuable avenue to elucidate factors involved in the establishment of the fasciation phenotype. The *f* locus was localized on LG 11 of the soybean conventional genetic map (Hedges et al., 1990). In soybean,

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several molecular maps have been generated using restriction fragment length polymorphism (RFLP), amplified restriction fragment length polymorphism (AFLP), and random amplified polymorphic DNA (RAPD) markers (Apuya et al., 1988; Lark et al., 1993; Akkaya, Bhagwat, and Cregan, 1995; Shoemaker and Specht, 1995; Keim et al., 1997; Cregan et al., 1999). However, none of these molecular maps contains the *f* locus.

We developed a population segregating for the fasciation phenotype. More than 700 markers (RFLPs with genomic or cDNA clones, AFLPs, and RAPDs) were used in mapping of the fasciation (*f*) locus. However, efforts to assign the *f* locus to the public soybean molecular map were not successful until simple sequence repeat (SSR) markers were used. Simple sequence repeat markers are single locus markers with multiple alleles. The SSR marker density exceeds 600 loci on the soybean molecular map (Cregan et al., 1999), thus facilitating information on recombination frequencies in the soybean genome.

In this study, we assigned the fasciation locus on LG D1b+W of the soybean molecular map. Positioning of the *f* locus is the first step toward a detailed genetic map of the region and physical isolation of this important developmental gene in soybean.

MATERIALS AND METHODS

Plant genotypes—The fasciation mutant line was a generous gift from a plant breeding company, Ring Around Research (Hale Center, Texas, USA). F_1 and F_2 progeny of crosses between the mutant line and three independent fasciation mutation sources, PI 83945-4, PI 243541, and T17, exhibited the fasciation phenotype, confirming that the same alleles were present in all lines (Tang and Skorupska, 1997). A mapping population consisting of 70 F_2 progeny was derived from a cross between cultivar Clark 63 and the fasciation mutant. The parental lines were polymorphic for flower color (*W1*) and seed coat pigmentation (*R*). The genotype of wild type Clark 63 was *W1W1RRFF* and the mutant was *w1w1rrff*. The F_2 genotypes of wild type individuals (homozygous or heterozygous) were determined by scoring the phenotype of the $F_{2:3}$ progeny lines.

DNA isolation and analysis—DNA was extracted from 4 g of young leaf tissue using the method described by Keim, Olson, and Shoemaker (1988). For bulk segregant analysis, 10 μg of DNA from each of ten homozygous *FF* F_2 progeny were pooled together for the *FF* bulk. Similarly, equal amounts of DNA from F_2 recessive genotypes were pooled to make the *ff* bulk. Restriction enzymes, *Eco*RI, *Eco*RV, *Dra*I, *Hin*dIII, *Taq*I, *Xho*I, and *Xba*I, were used individually to digest total genomic DNA of mutant and wild type and F_2 progeny DNA. Southern analysis with genomic and cDNA clones was performed as described previously (Skorupska et al., 1993).

Restriction fragment length polymorphism marker analysis—Almost 400 clones were used in RFLP analysis. Two hundred forty-nine soybean genomic clones were obtained from Biogenetic Services (Brookings, South Dakota, USA). The selected RFLP probes spanned the linkage groups of the public soybean molecular map at intervals of approximately 20 centimorgans (cM) (Cregan et al., 1999). In Southern analysis, the RFLP patterns of genomic clones obtained for Clark 63 and the fasciation mutant were compared with the patterns in SoyBase (http://soybase.agron.iastate.edu) for assignment of loci according to the public soybean molecular map.

One hundred thirty-one clones were isolated from a λZapII (Stratagene, LaJolla, California, USA) cDNA library constructed from shoot apices of the fasciation mutant at stages V3–V4 and differentially screened with cDNA from wild type and mutant epicotyls (Tang, 1999). For probe synthesis, inserts of λZapII cDNA clones were amplified by polymerase chain reaction (PCR) using T3 and T7 primers. The amplification conditions were: 1 min denaturation at 93°C; 30 cycles of 45 s at 93°C, 2 min at 60°C, and 3 min at 72°C; and 5 min at 72°C. The clones were labeled with 15 μCi [α-^{32}P] dCTP by random oligonucleotide-primed synthesis (Feinberg and Vogelstein, 1983).

Two cDNAs, *UnP2* and *UnP5*, were obtained using differential display analysis (GenHunter, Nashville, Tennessee, USA) of cotyledonary tissue of 5-d-old seedlings of mutant and wild type cDNA (Karakaya, 2001). The cDNA fragments were cloned into pGEM vector (Promega, Madison, Wisconsin, USA) and amplified with gene-specific primers: *UnP1* with forward primer 5′-GCTAACGCAAGGGCATCAGAGG-3′ and reverse primer 5′-TGTCCCTTCAGCGGGATCTTGTT-3′; *UnP5* with forward primer 5′-GATGAGGATGAGGACGATGATT-3′ and reverse primer 5′-CGAAGTAGCAAGACCATTCTCTCT-3′. A cytokinin oxidase cDNA, *GmCKOX*, was obtained by reverse transcriptase-polymerase chain reaction (RT-PCR) using 5′-GAGCAAGGCCCCATAAATAATTG-3′ and 5′-CAGGGATATAGGCTAGTCCTTGGAG-3′, forward and reverse primers, respectively (Karakaya, 2001). The cDNAs were labeled with [α-^{32}P] dCTP by PCR. The PCR labeling mixture contained 40 ng of template, 1 × PCR buffer (Perkin-Elmer, Norwalk, Connecticut, USA), 2 mmol/L $MgCl_2$, 0.4 mmol/L dATP, 0.4 mmol/L dGTP, 0.4 mmol dTTP, 2 pmol gene specific primers, 50 μCi [α-^{32}P] dCTP, and 1 unit Taq polymerase (Perkin-Elmer) in a 20-μL volume. The PCR conditions were denaturation at 94°C for 2 min; 30 cycles of 94°C for 1 min; annealing at 60°C for *UnP1*, 55°C for *UnP5*, or 57°C for *GmCKOX* for 40 s; 72°C for 1 min; and extension at 72°C for 5 min.

Sequencing—Clones were sequenced using the ABI Prism Dye Terminator Cycle Sequencing Ready Reaction DNA Sequencing Kit (Perkin-Elmer, Foster City, California, USA). Sequencing was performed using an ABI 373 Automated Sequencer (Model Version 2.1.1. Perkin-Elmer). Sequence analysis was conducted using BLASTX (http://www.ncbi.nlm.nih.gov/BLAST/). Additional sequence searches were conducted using expressed sequence tag sequences generated by The Soybean Expressed Sequence Tag (EST) Project, which is an excellent resource for functional and comparative analyses (R. C. Shoemaker, Project Leader; http://soybase.agron.iastate.edu). A MEGABLASTN program with the Est Others database field http://www.ncbi.nlm.nih.gov/BLAST/ was used for sequence comparisons using default parameters.

Amplified restriction fragment length polymorphism marker analysis—The AFLP analysis was performed as described by Vos et al. (1995) using an AFLP Analysis System I, AFLP Starter Primer Kit (Life Technologies, Gaithersburg, Maryland, USA). For DNA digestion, two restriction enzymes, *Eco*RI and *Pst*I, were used in combination with *Mse*I. A total of 64 *Eco*RI/*Mse*I primer combinations and 81 *Pst*I/*Mse*I primer combinations were tested in bulk segregant analysis with DNA samples of Clark 63, fasciation mutant, the F_2 *FF* bulk, and the F_2 *ff* bulk. Twenty-five primer combinations that either generated a high number of polymorphic fragments or fragments corresponding to the wild type pattern (Clark 63 and the F_2 *FF* bulk) vs. the mutant pattern (the fasciation mutant and the F_2 *ff* bulk) were tested in the F_2 progeny.

Random amplified polymorphic DNA marker analysis—The RAPD analysis was conducted using decamer primers (Operon Technologies, Alameda, California, USA). Amplification conditions used were as described in Skorupska et al. (1994). A total of 250 primers were tested on parental and the F_2 *FF* and F_2 *ff* DNA bulks. Forty-eight primers that produced distinct polymorphisms were tested in the F_2 population.

Simple sequence repeat marker analysis—LG 11 of the classical soybean map containing the *f* locus was integrated with LG D1b+W of the soybean molecular map (Cregan et al., 1999). Twenty-five SSR primer pairs (Satt005, Satt041, Satt069, Satt089, Satt135, Satt141, Satt172, Satt189, Satt198, Satt202, Satt271, Satt274, Satt282, Satt290, Satt350, Satt412, Satt428, Satt459, Satt506, Satt537, Satt546, Satt579, Satt600, Satt604, and Satt703) from LG D1b+W were obtained from the USDA-ARS, Soybean Genomics and Improvement Laboratory, Beltsville, Maryland, USA. The amplification of SSR was conducted as described in Cregan and Quigley (1997). Amplification products were fractionated on a 3% NuSieve 3 : 1 agarose gel (Bio

TABLE 1. Molecular marker polymorphism between Clark 63 and the fasciation mutant in soybean.

Parameter	Markers			
	RFLP	cDNA	RAPD	AFLP
Number probes/primers	249	134	250	145
Total fragments detected	1494	726	1750	8000
Polymorphic fragments	99	62	75	478
Percentage of polymorphic fragments	6.6	8.5	4.3	6.0

Whittaker Molecular Applications, Rockland, Maryland, USA) and stained with ethidium bromide for observation of SSR fragments.

Linkage analysis—Linkage analysis was conducted with Mapmaker/Exp 3.0 (Lander et al., 1987) using a logarithm of odds (LOD) score of 3.0. Recombination frequencies were converted to map distance in centimorgans by the Kosambi function (Kosambi, 1944).

RESULTS

Molecular polymorphism between Clark 63 and the fasciation mutant was investigated with more than 700 markers, including 249 genomic and 134 cDNA clones in RFLP analysis, 250 RAPDs, and 145 AFLP primer combinations. Genomic differences estimated by the number of polymorphic fragments out of the total detected fragments in Southern analysis with genomic probes was 6.6% and with cDNA clones was 8.5% (Table 1). In RAPD analysis, 4.3% of amplified fragments were polymorphic between Clark 63 and the fasciation mutant (Table 1).

In AFLP analysis, the number of DNA fragments generated by each primer combination ranged from 40 to 70, with an average of 55 distinct fragments per reaction. There were no obvious differences between the *Eco*RI/*Mse*I and the *Pst*I/*Mse*I primer combinations in the total number of fragments generated. The polymorphism frequency between wild type and mutant detected by *Eco*RI/*Mse*I was 7.2% compared with 4.9% for the *Pst*I/*Mse*I primer combination (data not shown). The difference may be due to the frequency of *Eco*RI sites in noncoding regions, which may have a higher level of genomic polymorphism.

In bulk segregant analysis, 14 AFLP markers had contrasting patterns between the *FF* genotypes (wild type and the *FF* F_2 bulk) and the *ff* genotypes (fasciation mutant and the *ff* F_2 bulk). Two of these markers, EaggMacc245 and EaacMcta250, were linked to the *f* locus based upon analysis of the F_2 population (Fig. 1). The association of these two AFLP markers with the *f* locus was further confirmed by the AFLP pattern of 26 *ff* F_2 segregants isolated from the independent F_2 population generated from Clark 63 and fasciation mutant cross (one recombinant occurred for marker EaacMcta250, and three recombinants were observed for marker EaggMacc245; data not shown). None of the polymorphic RAPD markers observed in bulk segregant analysis linked with the fasciation phenotype.

At an LOD score of 3.0, 241 loci were assigned to 31 LGs, spanning 3050 cM. All 20 LGs of the public molecular map were represented on the map constructed from the Clark 63 $\times$ the fasciation mutant F_2 population (Fig. 1). Eleven small linkage groups, spanning 509 cM, did not link with the public soybean molecular map (data not shown). The *W1* locus (flower color) mapped on LG F. The *R* locus (pigmentation of seed coat) mapped on LG K. The map assignments of these phenotypic markers are in agreement with a previous report (Shoemaker and Specht, 1995). Segregation of the fasciation phenotype gave a good fit to the expected 1 : 2 : 1 ratio based on $F_{2:3}$ genotypes (wild type : heterozygote : mutant = 19 : 35 : 16, $\chi^2 = 0.26$, $P > 0.75$). When using RFLP, AFLP, and RAPD markers, the fasciation phenotype was not assigned a locus on a linkage map. The successful positioning of the *f* locus was accomplished using SSR markers that facilitated detection of recombination events on LG D1b+W. Three SSR markers, Satt005, Satt141, and Satt600 linked with the *f* locus within 15 cM (Figs. 1 and 2). The order and map distances of the SSR loci differed from those reported by Cregan et al. (1999). A new locus, Satt703, mapped close to the terminal region of LG D1b+W permitting orientation of the linked AFLP and SSR markers and the *f* locus toward the proximal region of LG D1b+W (Fig. 1).

Seventeen RFLP markers obtained with cDNA clones mapped to 11 LGs of the public molecular map. Only cDNAs with similarity to known or unknown proteins were given locus assignments (Fig. 1). The GenBank accession numbers are presented in Table 2. We also used the soybean expressed sequence tag database (http://www.ncbi.nlm.nih.gov/blast/index.html) as a *Glycine max* species reference for clones isolated in our laboratory. The mapped clones encode housekeeping proteins such as: ribosomal protein subunits S8 (*Rps8*) and L31 (*RpsL31*) (mapped on LG G and M, respectively); histone protein (*H2B*) (mapped on LG B1); and several important development related proteins (Table 2). Five clones (*UnP1*, *UnP2*, *UnP3*, *UnP4*, *UnP5*) with similarities to *A. thaliana* unknown proteins mapped on LG A2, C2, K, or L (Table 2). Expressed sequence tag soybean database searches with the *UnP1*, *UnP2*, *UnP3*, *UnP4*, or *UnP5* sequences identified ESTs, confirming that the mapped clones function during vegetative or reproductive stages in soybean.

A vegetative storage protein (VSP27), a beta-amylase (BA, an exo-type starch hydrolyzing enzyme), and two unknown proteins (UnP1, UnP2) were assigned loci to LG A2 (Fig. 1). Positioning of the VSP27 confirmed the previous locus assignment to LG A2 (Shoemaker and Specht, 1995). The formin homology (FH) protein locus was mapped on LG C1. The FH proteins are implicated in cell polarization or cytokinesis through cytoskeletal function organization (Banno and Chua, 2000; Ozaki-Kuroda et al., 2001). *Eco*RI, *Dra*I, *Hin*dIII, and *Taq*I RFLP polymorphisms detected with the clone encoding PRC1, an IOTA subunit of the proteasome, mapped on LG C2, 0.7 cM from RFLP marker A397-1. A full-length PRC1 cDNA (994 bp) was isolated, and the sequence was submitted to GenBank (GBAN-AF034572). In plants, proteasomal subunits, including IOTA subunit, were shown to be related to cell division, suggesting that their role in the regulation of developmental events occurs by controlling the levels of regulatory proteins in proliferating tissues rather than protein degradation in tissue senescence (Ito et al., 1997; Bahrami and Gray, 1999).

Two cDNA clones, which were mapped on the LG F, encode for a highly conserved ubiquitin carboxyl-terminal hydrolase, UBPC, which is involved in ATP-dependent protein degradation (Viestra, Langan, and Haas, 1985) and putative cytokinin oxidase, *GmCKOX*. In the soybean EST database, which contains more than 189 000 sequences, several *CKOX* sequences are reported. *GmCKOX* in this study had sequence homology with GBAN-AW830743 (Table 2). The soybean

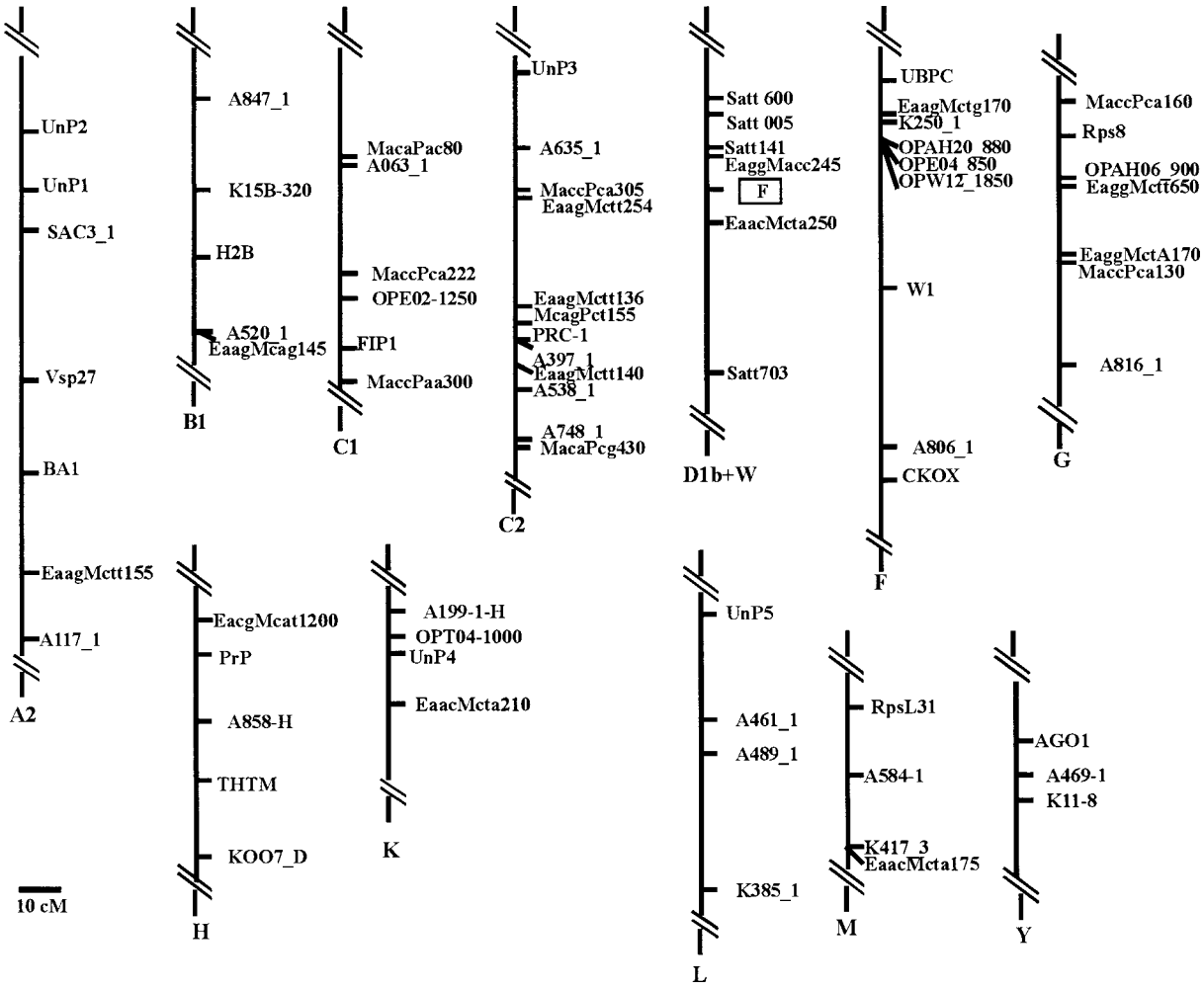

Fig. 1. Map position of the *f* locus and 17 cDNA marker loci on the linkage molecular map constructed from the Clark 63 × the fasciation mutant F_2 population. Linkage groups A to Y correspond to the USDA-ARS soybean molecular linkage map. Map distances are shown in centimorgans. The fasciation locus is boxed. Only the segments of the linkage groups containing the mapped cDNA loci are shown. AFLP loci: restriction enzymes are indicated by capital letter followed by anchor sequence in small letters and the size in base pairs; RAPD markers: primer is in capital letters followed by DNA fragment size in base pairs. Designation of cDNA marker loci: Vsp27 = vegetative storage protein, BA1 = beta-amylase, FIP1 = putative FH protein interacting protein FIP1, PRC1 = proteasome IOTA subunit, UBPC = putative ubiquitin carboxyl terminal hydrolase, CKOX = cytokinin oxidase, Rps8 = 40S ribosomal protein S8, PrP = putative pre-mRNA splicing factor, THTM = thioredoxin m-type chloroplast precursor, RpsL31 = 60S ribosomal protein L31, AGO1 = Argonaute1 protein, and UnP = unknown protein.

CKOX EST (GBAN-BG651837) was identified in a cDNA library constructed from mRNA isolated from reproductive shoot apices of the fasciation mutant, which was provided by our laboratory for the Soybean EST sequencing project. Isolation and characterization of the full-length cDNA of this new *CKOX* gene is in progress.

Two clones were mapped on LG H. These encode a pre-mRNA splicing factor (PrP) associated with the G0/G1 and G1/G2 transitions in the cell cycle and a chloroplast m-type thioredoxin precursor protein (THTM) involved in various redox reactions through the reversible oxidation (Lopez et al., 1994).

Argonautel (AGO1) belongs to a novel class of proteins controlling leaf, floral organ, and axillary meristem development in *Arabidopsis* (Bohmert et al., 1998; Lynn et al., 1999). In RFLP analysis, the soybean clone GBAN-B1740284 with similarity to Argonautel showed independent assortment from the fasciation locus and mapped on LG Y of the soybean molecular map.

DISCUSSION

In this study, different marker systems, including RFLP, RAPD, AFLP, and SSR, were used for mapping the fasciation locus in soybean. Due to the limited germplasm sources used in the development of soybean cultivars, polymorphism in *Glycine max* is generally low (Shoemaker and Specht, 1995). The estimation of the genome diversity between Clark 63 and the fasciation mutant by RFLP and AFLP markers was less than 10%. The mechanisms of RFLP and AFLP are based on the variation of nucleotides at restriction sites of homologous sequences between two genomes, and it is not surprising to find a similar estimation of genomic diversity using these two methods. The lower polymorphism (4.3%) between Clark 63 and the fasciation mutant estimated with RAPD markers might

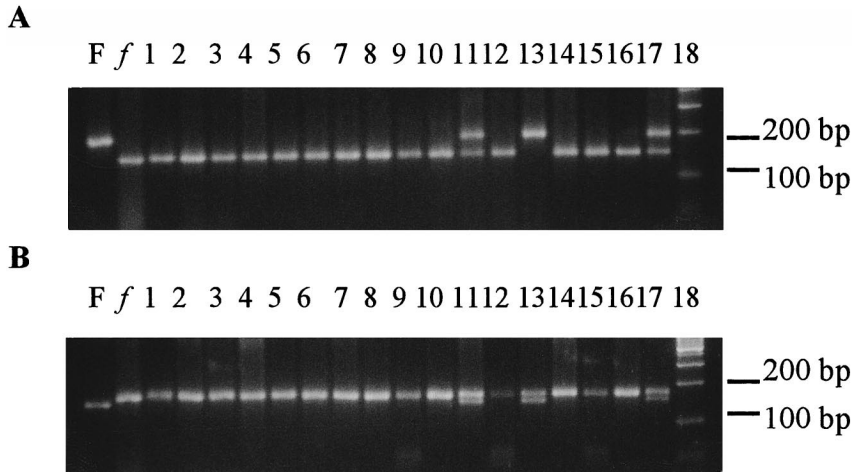

Fig. 2. Simple sequence repeat marker polymorphisms detected (A) with Satt141 and (B) Satt005 and linked with the fasciation (*f*) locus on linkage group D1b+W of the molecular map constructed from the Clark 63 × the fasciation mutant F_2 population. Order of genotypes: Clark 63 (F), fasciation mutant (*f*), 1–17 are F_2 progeny expressing the fasciation phenotype. Progeny 11 and 17 are heterozygotes for Satt141 and Satt005 markers. Polymerase chain reaction products were separated in 3% NuSieve 3 : 1 agarose gel. Lane 18 is 100 bp DNA ladder (Promega, Madison, Wisconsin, USA).

be due to low annealing temperature used in RAPD analysis that allows some mismatching and thereby can mask some of the nucleotide variation between the two genomes.

We observed an overall expansion of genetic distances on the Clark 63 × the fasciation mutant map relative to those on the soybean molecular map of Cregan et al. (1999). Progeny number or different recombination frequencies in a particular cross might affect overall scores of genetic distances. Genomic similarities between Clark 63 and the fasciation mutant could contribute to higher recombination frequencies and thus map expansion, including LG D1b+W on which the fasciation locus was mapped. Jin et al. (1998) and Hegstad et al. (2000) also reported expansion of genetic distances on soybean LG D1b+W in mapping of a male-sterile gene and the *wp* flower color locus, respectively. Hegstad et al. (2000) speculate that increased recombination rates in the LG D1b+W region may be caused potentially by rearrangements or insertions generated by a defective *wp-m* transposable element. Linkage of the *f* locus and the *wp* locus with Satt141 and Satt600 on the LG D1b+W suggests that further investigations of the region might elucidate the origin of gene mutation and structural rearrangements of that region of the soybean genome.

An advantage of using cDNA clones for mapping is the identification of actively transcribed regions of the genome. In combination with sequence data, mapping of cDNAs enhances the characterization of gene distribution on each chromosome and within the whole genome. Seventeen cDNAs were mapped to 11 different linkage groups of the public soybean molecular map, adding to previously mapped cDNAs: histone H3 genes (Kanazin, Blake, and Shoemaker, 1996), disease resistance gene analogs (Kanazin, Fredrick, and Shoemaker, 1996; Yu, Buss, and Maroof, 1996), chalcone synthase multigene family (Todd and Vodkin, 1996), β-1,3-glucanases genes (Jin et al., 1999), cDNAs from various studies (Matthews et al., 2001),

TABLE 2. Soybean cDNA clones with assigned loci on the linkage map constructed from the F_2 population of Clark 63 × the fasciation mutant.

Linkage group	Clone designation	BLASTX search: Identified accessions	BLASTX search: Accession number[a]	Soybean cDNA accession number
A2	Vsp27	*Glycine max*, vegetative storage protein VSBP	GBAN-P10743	GBAN-BI740272
	BA1	*Glycine max*, beta-amylase	GBAN-BAA09462	GBAN-BI740273
	UnP1	*Arabidopsis thaliana*, unknown protein	GBAN-BAB02406	GBAN-BI740274
	UnP2	*Arabidopsis thaliana*, unknown protein	GBAN-AAD10648	GBAN-AF349572
B1	H2B	*Gossypium hirsutum*, histone H2B	GBAN-O22582	GBAN-BI740275
C1	FIP1	*Arabidopsis thaliana*, putative FH protein interacting protein FIP1	GBAN-AAF14549	GBAN-BI740276
C2	UnP3	*Arabidopsis thaliana*, unknown protein	GBAN-AAG12540	GBAN-BI740277
	PRC1	*Glycine max*, 20S proteasome IOTA subunit	GBAN-AAC28135	GBAN-AF034572
F	UBPC	*Arabidopsis thaliana*, putative ubiquitin carboxyl-terminal hydrolase	GBAN-BAB11409	GBAN-BI740278
	CKOX	*Arabidopsis thaliana*, putative cytokinin oxidase	GBAN-BAA97027	GBAN-AW830743
G	Rps8	*Oryza sativa*, 40S ribosomal protein S8	GBAN-P49199	GBAN-BI740279
H	PrP	*Arabidopsis thaliana*, putative pre-mRNA splicing factor	GBAN-AAD11585	GBAN-BI740280
	THTM	*Pisum sativum*, thioredoxin m-type chloroplast precursor	GBAN-P48384	GBAN-BI740281
K	UnP4	*Arabidopsis thaliana*, unknown protein	GBAN-BAB16471	GBAN-BI740282
L	UnP5	*Arabidopsis thaliana*, unknown protein	GBAN-AAF04419	GBAN-AF350328
M	RpsL31	*Panax ginseng*, 60S ribosomal protein L31	GBAN-BAA96368	GBAN-BI740283
Y	AGO1	*Arabidopsis thaliana*, Argonaute1 protein	GBAN-004379	GBAN-BI740284

[a] The prefix GBAN- has been added to each accession to link the online version of *American Journal of Botany* with GenBank but is not part of the actual accession number.

and genes associated with cyst nematode infection of soybean (Vaghchhipawala et al., 2001).

Mapping of cDNA markers may allow identification of genes corresponding to classical loci and association with phenotype. However, mutations caused by single-base changes or frame shifts are difficult to detect by RFLP analysis unless the restriction sites used are altered by the mutation. Several of the mapped clones were differentially expressed between the mutant and Clark 63 (Tang, 1999; Karakaya, 2001). None of the tested cDNA markers mapped at the fasciation locus suggesting that several genomic regions might be involved in the establishment of the fasciation mutant phenotype. *GmCLV1* and *GmFAS2*, orthologs of *Arabidopsis* genes that cause a fasciation phenotype, were excluded as candidate genes for fasciation mutation in soybean based on expression and mapping analyses (Yamamoto, Karakaya, and Knap, 2000; Karakaya, 2001). Another important soybean clone with similarity to Argonaute1 (mutation in *Argonaute1* pleiotropically affects development of apical shoot meristem, axillary meristems, and leaves) mapped to LG Y. Also, *GmCKOX* showed independent assortment from the fasciation phenotype and mapped to LG F. Cytokinin oxidase inactivates cytokinins by irreversible degradation (Kaminek, Motyka, and Vankova, 1997), which could be one of the possible mechanisms for differences in cytokinin levels observed between the mutant and the wild type (Karakaya, 2001). Characterization of the second *GmCKOX* identified by the EST sequencing of a cDNA library from reproductive shoot apices of the fasciation mutant might aid information on association of cytokinin oxidase genes with the fasciation mutant phenotype.

Assignment of the *f* locus to linkage group D1b+W and identification of flanking markers can be used as an initial step for precise mapping of the *f* gene. It is likely that the *f* locus product acts upstream of other genes in soybean development and intervenes directly or indirectly in several signal transduction pathways throughout vegetative and reproductive development. Hence, the mutant can be a valuable resource for investigation of genomic organization, regulation of soybean developmental traits, and evolution of key morphological characters.

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